Lecture 23: Regeneration of Myocardium

Prof. Thomas Groth

Biomedizinische Materialien

Martin Luther University Halle-Wittenberg
Content

• Structure and function of heart muscle (myocardium)
• Electromechanical coupling of cardiomyocytes
• Approaches of conventional treatment of myocardium infarct
• Survey in tissue engineering of hearth muscle
• Origin and manipulation of cells
• Example of animal models and some first clinical studies
Heart Anatomy and Function

De-oxgenated blood flows from the vein A in the right atrium B, which pumps it through the tricuspidal valve C into right ventricle D. There it is ejected through the Pulmonal valve E in the lung artery F and then into the lung. The oxygenated blood flows from the lung vein G in the left atrium H. From there it is pumped through the Mitral valve I in the left ventricle J. The left ventricle ejects the blood through the aorta valve K in the Aorta curvature L and then in the aorta M.
Microscopical Anatomy of Myocardium

InterActive Physiology®: Cardiovascular System: Anatomy Review: The Heart
Normal Structure of Heart Muscle

Cardiomyocytes with cross-striated structure by myofibrills and centrally located nuclei,

Arrows: electromechanical coupling of cells by Glanzstreifen

http://alf3.urz.unibas.ch/pathopic/getpic-fra.cfm?id=4108
Cardiovascular Diseases

- Atherosclerosis → chronic, progressive, multifocal disease of wall of blood vessels with formation of atherosclerotic plaque
- Hearth infarction – ischemia of parts of heart muscle by narrowing or occlusion of arteries
- Aneurysm → weakening and dilatation of vessel wall with reduced wall thickness and risk of vessel rupture
- Stenosis of heart valves after endocarditis
- etc.

Percentage of persons in Germany who take pharmaceuticals for treatment of cardiovascular diseases

Daily dose of pharmaceuticals for treatment of cardiovascular diseases with age
Cardiovascular Diseases

Death rates from Coronary Heart Disease among Nations per 100 000 population aged 35-74

Deaths per 100 000 population

- Ireland
- Scotland
- South Africa
- England
- USA
- Netherlands
- Austria
- Italy
- France
- Japan

cardiologydoc.wordpress.com
Atherosclerotic Plaque with Thrombus Formation after Rupture

L – lumen of blood vessel, F- fibrotic tissue, C – cholesterol crystals

Myocardial Infarction (MI)

- Temporary or permanent ischemia of heart muscle → hypoxia and necrosis of cardiomyocytes
- Cardiomyocytes terminally differentiated cells → Inability to divide
- After MI sequence of events → acute inflammation, granulation tissue, formation of scar tissue
- Reduced performance of heart muscle (cardiac output) and arrhythmias
- Immediate or later heart failure if loss of cardiomyocytes was large → chronic pathological remodelling of left ventricle (hypertrophia)
- Formation of heart aneurysma with rupture of heart wall or septum
Myocardium Infarction - Histology

Necrotic cardiomyocytes (arrows) → loss of nuclei, presence of granulocytes (stars) → digestions of dead cells (inflammation); monozytes → growth factors (scarring)

http://alf3.urz.unibas.ch/pathopic/getpic-fra.cfm?id=814
Histology of Scar Tissue
Scar Tissue - Macroscopically

- Replacement of myocardium by connective tissue
- No contractile activity
- No involvement in nerve guidance – arrhythmias possible
- Smaller wall thickness and decreased mechanical strength
- Aneurysma formation possible

www.mirm.pitt.edu/news/article.asp?qEmpID=46
Conventional Treatment of MI

- Immediate removal of occlusion or narrowing by PCTA → Balloon dilatation followed by stenting
- Thrombolytic therapy with streptokinase, urokinase, tissue plasminogen activator, etc.
- Bypass surgery to improve blood supply
- Pharmaceutical treatment to improve performance
- Hearth pacemaker to treat arrhythmias, bradycardia or ventricular fibrillation
- Left heart assist systems
- Heart transplantation

→ So far only very limited options to foster regeneration of myocardium
PTCA – Percutaneous Transluminal Coronary Angioplasty + Stents
X-Ray Image of Coronary Artery Before and After PTCA

Fig 3: Severe stenosis in a coronary artery (top), and angiographic appearance (bottom) after stent deployment.
Attempts to Regenerate Myocardial Tissue

- Direct transplantation of (stem) cells in infarcted tissue
- Regenerative therapies of myocardial tissue with cytokines (in vivo)
- Tissue Engineering of myocardium -analogous tissue constructs
Cell Therapy of Myocardial Infarction

• Re-colonisation of infarcted area with cells → Minimising scar tissue formation and replacement of necrotic tissue

• Fetal cardiomyocytes, skeletal muscle cells and stem cells from bone marrow → limited but measurable ability of myocardial and improved cardiac output

• Problems:
  - Cell death after transplantation,
  - No differentiation of cells into cardiomyocytes
  - No functional integration into myocard,
  - No electromechanical coupling between cells

Example: Preclinical Tests with ESC

- Kofidis et al. 2004
- Rats as animal model
- ESC labelled with „green fluorescent protein“ (GFP)
- Cultivation of ECS in media cardiotropic factors
- Experimental induction of infarct by ligation of coronary artery
- Mixing of cells with matrigel during injection into myocard → Matrigel as matrix for improved engrafting
- Detection of connexin 43 and α-sarcomer-aktin in GFP+cells
Preclinical Studies (Rats)–Engrafting of Matrigel Injected ESC in Infarcted Myocard

Homogenous distribution of ESC in scar tissue (nuclei stained blue with DAPI blue staining)

Engrafted cells (green – GFP) express connexin (yellow)
Effect of Matrigel Injected ESC on Cardiac Output and Ventricle Thickness

• Estimation of heart function by echocardiography → End-Systolic-Diameter (ESD); End-Diastolic-Diameter (basal and apical), Fractionated shortening of heart FS = (EDD-ESD)/EDD

• Estimation of heart wall thickness and septum thickness
Results Echocardiography I

HTX – heart transplantation
LAD – left anterior descending artery
Results Echocardiography II

Wall thickness    Septum thickness
Direct Transplantation of HSC in Humans
University of Rostock Prof. Steinbrück

• Use of CD 133+ cells → hematopoietic stem cells (HSC) with high plasticity

• Aspiration of bone marrow of patients with acute MI

• Isolation of CD 133+ cells with magnetic cell separation

• Injection of CD 133+ cells in infarcted area
Injection of Stem Cells in Myocard
Improved Blood Supply to Infarcted Myocardial Area & Heart Performance After Stem Cell Injection

MRT images of patient before (left) and after (right) stem cell injection

Indication for better blood supply

LVEF – Left ventricular ejection fraction – measure for blood transport of left ventricle

All data from Steinhoff group, University of Rostock
Induction of Homing of Stem and Progenitor Cells from Bone Marrow to Cardiac Muscle

Localized SDF-1alpha gene release mediated by collagen substrate induces CD117+ stem cell homing

Weiwei Wang a, #, Wenzhong Li a, #, Lee-Lee Ong a, Dario Furlani a, Alexander Kaminski a, Andreas Liebold a, Karola Lützow b, Andreas Lendalein b, Jun Wang c, Ren-Ke Li d, Gustav Steinhoff a, Nan Ma a, b, *

a Department of Cardiac Surgery, University of Rostock, Germany
b Institute of Polymer Research, GKSS Forschungszentrum, Germany
c National Laboratory for Physical Sciences at Microscale, University of Science and Technology of China, China
d Division of Cardiovascular Surgery, Toronto General Hospital and the University of Toronto, Canada
In Vitro Transfection of Cells with Stromal Cell Derived Factors SDF-1

Plasmid pEGFP-N3-SDF-1 + Polyethylene imine – nanoparticles

Embedding of nanoparticles in collagen gel → transfection system

Seeding of stromal cells → transfection with SDF-1

Expression and shedding of SDF-1, GFP+

(Peripheral blood) containing CD 117+ multipotent stem cells → attracted and immobilised by SDF-1

Collagen gel

TE scaffold material
Fig. 6 \textit{In vitro} statistic analysis of migration and homing of CD117$^+$ cells. (A) Flow chamber employed to simulate the circulating environment. (B) Representative FACS histogram of CD117$^+$ cells purity assay. (C) The number of CD117$^+$ cells on the GAC-coated area normalized by the number of COS7 cells. (D) EGFP expression of COS7 cells cultured on the GAC (PEI/pEGFP-N3-SDF-1$\alpha$/collagen)-coated area. (E) a CD117$^+$ cell labelled with CellTracker Probe-Red CMTPX. (F) Merged picture of fluorescence and phase-contrast microscopic picture and (G) enlarged 3D-picture that indicates a homed CD117$^+$ cell on a transfected COS7 cell. (Bars in D–F = 30 $\mu$m; Bar in G = 10 $\mu$m).
Tissue Engineering Myocard

J. Leor et al. / Pharmacology & Therapeutics 105 (2005) 151–163
Approaches of in Vitro TE Myocardial Tissue

I. Implantation of cells in scaffold from synthetic or biological materials → „Artificial Myocardial Tissue“ (AMT)

II. Embedding of cells in extracellular matrix material (e.g. collagen) → “Engineered Heart Tissue“ (EHT)

III. Fusion of cell monolayers to suprastructures
Approaches of in Vitro TE Myocardial Tissue

Collagen
Alginate
PGA
PLA

Collagen + ECM

Cardiac myocyte monolayers

I
II
III
# Used Materials TE Myocardial Tissue

## Overview of applied methods in cardiac tissue engineering

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Constitution</th>
<th>Bioreactor</th>
<th>Study</th>
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<td>Polystyrene beads/collagen threads</td>
<td>Solid</td>
<td>+</td>
<td>[6]</td>
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<tr>
<td>Polyglycolic acid</td>
<td>Solid</td>
<td>+</td>
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<td>Gelatine mesh</td>
<td>Solid</td>
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<tr>
<td>Modified collagen</td>
<td>Solid</td>
<td>±</td>
<td>[5]</td>
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<tr>
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<tr>
<td>Collagen mesh</td>
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<td>[2]</td>
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<tr>
<td>Collagen</td>
<td>Liquid/gel</td>
<td>+</td>
<td>[8]</td>
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<tr>
<td>Collagen/matricegel</td>
<td>Liquid/gel</td>
<td>+</td>
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Requirements to Obtain Functional Tissue

- Large number of cells
- Improved survival rates of cells in 3-D-constructs
- Sufficient dimensions of the construct
- Sufficient contractile forces
- Sufficient supply of oxygen after implantation (neovascularisation)
Cells

- 1 g Myocard $\rightarrow$ ca. 20 – 40 Mio. Cardiomyocytes

- Typical Myocard infarct $\rightarrow$ Loss of about 50 g tissue $\rightarrow$ ca. 1 – 2 Mrd. Cells

- Problem: Source for sufficient number of cells!

- Clonal growing embryonic or adult stem cells as source?
Sources for Cells TE Myocard

- Requirements: simple isolation, ease of growth, non-immunogenic, differentiation to cardiomyocytes → So far not all requirements fulfilled!

Table 1
Potential cell sources for myocardial tissue engineering

1. Fetal cardiomyocytes (Li et al., 1999; Leor et al., 2000)
2. Skeletal myoblasts (Kamelger et al., 2004; Li, 2004)
3. Mesenchymal stem cells (Krupnick et al., 2001)
4. Smooth muscle cells (Matsumarashi et al., 2003)
5. Endothelial progenitor cells (Wu et al., 2004)
6. Crude bone marrow (Ryu et al., 2005)
7. Umbilical cord cells (Kadner et al., 2004)
8. Fibroblasts (Li et al., 2000; Kellar et al., 2001)
9. Human embryonic stem cells (Levenberg et al., 2003)
10. Cloned cells (Lanza et al., 2004)
### Cells TE Myocard

<table>
<thead>
<tr>
<th>Cells Type</th>
<th>Autologous</th>
<th>Easily Obtainable</th>
<th>Highly Expandable</th>
<th>Cardiac Myogenesis</th>
<th>Clinical Experience</th>
<th>Safety Concerns</th>
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<td>Fetal cardiomyocytes</td>
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<td>No</td>
<td>No</td>
<td>Yes</td>
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<td>Embryonic stem cells</td>
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<td>No</td>
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<td>Yes</td>
<td>No</td>
<td>Yes teratoma</td>
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<tr>
<td>Skeletal myoblasts</td>
<td>Yes</td>
<td>Yes</td>
<td>Depend on age</td>
<td>Debated</td>
<td>Yes</td>
<td>Yes arrhythmias</td>
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<tr>
<td>Crude bone-marrow cells</td>
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<td>Yes</td>
<td>Depend on age</td>
<td>Debated</td>
<td>Yes</td>
<td>Yes calcification</td>
</tr>
<tr>
<td>Mesenchymal stem cells</td>
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<td>No</td>
<td>Depend on age</td>
<td>Yes</td>
<td>No</td>
<td>Yes Fibrosis calcification</td>
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<tr>
<td>Hematopoietic stem cells</td>
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<td>Yes</td>
<td>Debated</td>
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<td>Yes</td>
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<tr>
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<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Smooth muscle cells</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
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</tr>
</tbody>
</table>
Functionality $\rightarrow$ Contractile Forces

- Normal myocardial tissue $\rightarrow$ Contractile forces per area up to 56 mN/mm²

- AMT - Contractile forces 0.02 mN/mm² $\rightarrow$ Limited organisation of cells in 3-D-constructs, little coupling between cells, massive scaffold from synthetic polymers (e.g. fibres) represent mechanical resistance

- EHTs und stacked monolayers (see appendix) 2 – 4 mN/mm² $\rightarrow$ problem assessment of „active“ cross section areas (passive matrix)

- EHT (0.3 mm²) $\rightarrow$ normalized force about 13 mN/mm²
Engineered Heart Tissue“ (EHT)

• Prof. Eschenhagen Universitätsklinik Hamburg-Eppendorf
• Embedding of neonatal rat cardiomyocytes in gel from collagen I and matrigel + growth factors
• Production of ring-shaped scaffolds
• Cultivation in vitro under cyclical mechanical stress
• Production of suprastructures from single rings
Engineered Heart Tissue™ (EHT)

- Prof. Eschenhagen Universitätsklinik Hamburg-Eppendorf
Contractile Activity in Vitro
Implantation Infarction Model: Rat

- Implantation into peritoneum or on top of rat heart
- Immune supression
- EHT vital und contractile over 8 weeks
Engineered Heart Tissue

- Remodelling of transplant within 4 weeks
- (b) Formation of thick heart muscle on infarcted region
- (c) larger magnification → Orientation of muscle fibres in the transplant
Preparation of Fused Monolayers (Sheets) of Cardiomyocytes

• Shimizu et al. 2003 (Okano’s lab)

• Application thermoresponsive polymers → Poly-N-isopropylmethacrylamide

• Polymers at 37°C hydrophobic → adsorption adhesive proteins, cell adhesion and growth

• Polymers below bei 32°C extremely hydrophilic (hydrogel) → no protein adsorption → detachment of cells
Effect of Thermoresponsive Polymers on Cell Adhesion

Enzymatic digestion by trypsin or other proteinases

37°C

20°C
Preparation of Cell Monolayer with Thermoresponsive Polymers

37°C

Cell-to-cell junction

37°C

Hydrophobic

ECM

(A)

20°C

Hydrophilic

(B) + trypsin

(C)
TE Myocardium with Cell Sheets

Electrical Coupling of Monolayers

Sheet A

Sheet B

Sheet A

Sheet B

1 sec
Anatomy + Histology of Fused Cell Sheets

Formation of new blood vessels in transplanted cell sheets (left) and formation of stratified cardiac tissue with blood vessels (right, arrows)
Literature

• W.-H. Zimmermann et al., Cardiovascular Research 2006, im Druck, (Review)

• Leor et al. Pharmacology & Therapeutics 105 (2005) 151-163, (Review)

• Zimmermann et al., Biomaterials 25 (2004) 1639 – 1647

• Shimizu et al. Biomaterials 24 (2003) 2309-2316